Claims

1. (Previously presented) A method of comparing the phosphorylation states of one or more proteins in two or more samples comprising:

providing a substantially chemically identical and differentially isotopically labeled protein reactive reagent for each sample wherein the protein reactive reagent satisfies the formula:

B-L-PhRG

wherein B is a binding agent, L is a linker group having one or more atoms that are differentially labeled with one or more stable isotopes, and PhRG is a phosphate reactive group that selectively reacts with amino acid residues of the one or more proteins that were formerly phosphorylated;

reacting each sample with one of the protein reactive reagents to provide proteins bound to the protein reactive reagent, whereby such bound proteins are differentially labeled with stable isotopes;

capturing bound proteins of the samples using a capture reagent that selectively binds the binding agent of the protein reactive reagent;

releasing captured bound proteins from the capture reagent by disrupting the interaction between the binding agent and the capture reagent;

detecting the amount of released bound proteins; and

comparing the amount of released bound proteins from one sample to the amount of released bound proteins from one or more other samples.

2. (Original) The method of claim 1, wherein the bound proteins in the samples are enzymatically or chemically processed to convert them into bound peptides.

3. (Original) The method of claim 1, wherein a protein portion of one or more of the bound proteins are sequenced by tandem mass spectrometry to identify the bound protein.

4. (Original) The method of claim 1, wherein the amount of one or more phosphorylated proteins in the sample is determined by mass spectrometry and further comprising introducing into a sample a known amount of one or more internal standards for each protein to be quantified.

5. (Original) The method of claim 1, wherein one or more phosphorylated amino acid residues are selected from the group consisting of threonine, serine, and tyrosine.

6. (Original) The method of claim 1, wherein the released bound proteins are separated by chromatography prior to detecting the bound proteins by mass spectrometry.

7. (Previously presented) The method of claim 1, wherein a plurality of proteins are detected and identified in one or more of the two or more samples.

8. (Previously presented) The method of claim 3, wherein all of the proteins in one or more of the two or more samples are identified.

9. (Previously presented) The method of claim 1, wherein the two or more samples are combined after being reacted with a protein reactive reagent and before the bound proteins of the samples are captured.

10. (Original) The method of claim 1, wherein the proteins being quantified are membrane proteins.

11. (Original) The method of claim 1, wherein different samples contain proteins originating from different organelles or different subcellular fractions.

12. (Previously presented) The method of claim 9, wherein each of the two or more samples are taken at different times, or contain proteins expressed in response to different environmental or nutritional conditions, or different chemical or physical stimuli.

13. (Original) The method of claim 1, wherein the different samples represent proteins expressed in different disease states.

14. (Original) A method for screening for a therapeutic that alters a phosphorylation state of a protein, the method comprising:

contacting at least one test sample containing the protein with the therapeutic;

providing at least one control sample containing the protein;

removing one or more phosphate groups from one or more amino acid residues of the protein in the at least one test sample and the at least one control sample;

tagging the at least one test sample and the at least one control sample with substantially chemically identical and differentially isotopically labeled protein reactive reagents for each sample, wherein the protein reactive reagents satisfies the formula:

B-L-PhRG

wherein B is a binding agent that selectively binds to a capture reagent, L is a linker group having one or more atoms that are differentially labeled with one or more stable isotopes, and PhRG is a phosphate reactive group that selectively reacts with amino acid residues that were formerly phosphorylated; and

detecting a level of phosphorylation of the tagged proteins in the at least one test sample and the at least one control sample; and

determining whether the therapeutic altered the level of phosphorylation of the tagged proteins in the at least one test sample.

15. (Withdrawn) A reagent for mass spectrometric analysis of proteins that satisfies the general formula:

B-L-PhRG

where B is a binding agent that selectively binds to a capture reagent, L is a linker group that comprises at least one isotopically heavy atom and a phosphorylation reactive group (PhRG) that selectively labels proteins at one or more residues that were formerly occupied by phosphate groups.

16. (Withdrawn) The reagent of claim 15, wherein PhRG is selected from the group consisting essentially of primary amines, secondary amines, tertiary amines, lactams, amides, imides, hydroxylamines, hydrazides, hydrazines, sulfites, sulfinates, sulfonamides, and mixtures thereof.

17. (Withdrawn) The reagent of claim 15 wherein the isotopically heavy atom is selected from the group consisting of ^{2}H, ^{13}C, ^{15}N, ^{17}O, ^{18}O, and ^{34}S.

18. (Withdrawn) The reagent of claim 15, wherein the reagent is soluble in a liquid protein sample.

19. (Withdrawn) The reagent of claim 15, wherein the linker is a cleavable linker.

20. (Withdrawn) The reagent of claim 15, wherein the binding agent is biotin or a modified biotin.

21. (Withdrawn) The reagent of claim 15, wherein the binding agent is selected from a group consisting essentially of a 1,2-diol, glutathione, maltose, a nitrilotriacetic acid, or an oligohistidine.

22. (Withdrawn) The reagent of claim 15, wherein the affinity label is a hapten.

23. (Withdrawn) A reagent for mass spectrometric analysis of proteins that satisfies the general formula:

$B\text{-}B^{1}\text{–}X^{1}\text{-}(CH_2)n\text{-}[X^{2}\text{-}(CH_2)m]_x\text{-}X^{3}\text{-}(CH_2)p\text{-}X^{4}\text{-}B^{2}\text{-}PhRG$

where: B is a binding agent, PhRG is a phosphate reactive group, $B^{1}\text{–}X^{1}\text{-}(CH_2)n\text{-}[X^{2}\text{-}(CH_2)m]_x\text{-}X^{3}\text{-}(CH_2)p\text{-}X^{4}\text{-}B^{2}$ is a linker group, wherein: X^{1}, X^{2}, X^{3} and X^{4}, are independently selected from a group consisting essentially of O, S, NH, NR, NRR^{1}+, CO, COO, COS, S-S, SO, SO_2, CO-NR, CS-NR^{1}, Si-O, aryl or diaryl, wherein at least one of the X^{1}, X^{2}, X^{3} and X^{4} groups comprises an isotopically heavy atom.

24. (Previously presented) A method of detecting different types of phosphorylated amino acid residues in one or more proteins, the method comprising:

providing one or more samples containing one or more proteins;

removing the phosphate group from at least one serine residue or at least one threonine residue of at least one protein in each sample;

removing the phosphate group from at least one tyrosine residue of at least one protein in each sample;

tagging the at least one serine residue or the at least one threonine residue with substantially chemically identical and differentially isotopically labeled protein reactive reagents for each sample, wherein the protein reactive reagents satisfies the formula:

B-L-PhRG

wherein B is a binding agent that selectively binds to a capture reagent, L is a linker group having one or more atoms that are differentially labeled with one or more stable isotopes, and PhRG is a phosphate reactive group that selectively reacts with amino acid residues that were formerly phosphorylated;

tagging the at least one tyrosine residue with substantially chemically identical and differentially isotopically labeled protein reactive reagents for each sample, which are differentially isotopically labeled relative to the protein reactive reagents used to tag the at least one serine residue of the at least one threonine residue, wherein the protein reactive reagents satisfies the formula:

B-L-PhRG

wherein B is a binding agent that selectively binds to a capture reagent, L is a linker group having one or more atoms that are differentially labeled with one or more stable isotopes, and PhRG is a phosphate reactive group that selectively reacts with amino acid residues that were formerly phosphorylated; and

detecting the tagged amino acid residues.

25. (Original) The method of claim 24, wherein the removing the phosphate group from at least one serine residue or at least one threonine residue is after the removing the phosphate group from at least one tyrosine residue.

26. (Original) The method of claim 24, wherein tagging the at least one serine residue or the at least one threonine residue is done after the tagging the at least one tyrosine residue.

27. (Original) The method of claim 26, wherein the removing the phosphate group from at least one serine residue or at least one threonine residue is after the removing the phosphate group from at least one tyrosine residue.